

A new chiral electrochemical sensor for the enantioselective recognition of penicillamine enantiomers

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Abstract A new chiral electrochemical sensor has been successfully prepared through chemical linking L-methotrexate (L-Mtx) onto the gold electrode surface. Cyclic voltammetry and electrochemical impedance spectroscopy were used to investigate the enantioselective interaction between L-Mtx and Pen enantiomers. The results showed that the L-Mtx-modified gold electrode can selectively recognize penicillamine (Pen) enantiomers using Zn(II) as central ion, and larger response signal was observed from D-Pen owing to the selective formation of Zn complexes. The interaction time between the modified electrode and Pen enantiomers containing Zn(II) was considered. And the electrochemical response of the modified electrode to a series of different concentration of Pen in the presence of Zn(II) was also monitored. In addition, the enantiomeric composition of D- and L-Pen enantiomer mixtures was monitored by measuring the current responses of the sample.

Keyword L-methotrexate-modified gold electrode · Chiral recognition · Zinc ions · Penicillamine enantiomers

Introduction

Many biological events are greatly influenced by the chirality because a great of drugs, food additives, and other

molecules own chirality [1, 2]. And the body exhibits different physiological responses to different enantiomers. One isomer may produce the desired therapeutic activities, while the other may be inactive or exhibit serious side effects [3]. For example, the R-enantiomer of thalidomide had pain relieving effect while the S-enantiomer was the cause of serious deformities in unborn children [4]. Research on enantiomeric recognition of chiral compound can provide important information to understand the recognition progress in biological systems. Although many efforts have been devoted to designing efficient systems for the enantioselective recognition of chiral molecules, there still remain many challenging tasks. Therefore, developing effective methods for chiral analysis is an attractive work and a research focus. There are many techniques developed for chiral analysis, including high-performance liquid chromatography [5–7], capillary electrophoresis [8, 9], quartz crystal microbalance [10, 11], fluorescence detection [12, 13], nuclear magnetic resonance protocols [14], and electrochemical methods [15, 16]. Among these approaches, electrochemical methods, an important and powerful analytical tool, have attracted considerable attention [17–19]. Such as, chiral PAMAM dendrimers G4.0-modified electrode has been used for the enantioselective recognition of alanine [20], chiral salen Mn(II) compound as ionophore for enantioselective potentiometric sensing of L-MA [21].

Penicillamine (Pen) is a thiol amino acid which is naturally occurring and has many therapeutic applications [22]. The D- and L-enantiomeric forms have different biological and toxicological properties. As a chiral drug, D-Pen is used in the treatment of Wilson's disease, hepatitis, certain forms of metal intoxication, and progressive systemic sclerosis [23]. It also can be used as crucial intermediate in pharmaceutical synthesis [24]. In contrast, L-Pen has considerable toxicity [25]. Therefore, the selective discrimination of Pen

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enantiomers is very important in the field of pharmaceutical sciences and biochemistry.

To achieve the goal of chiral recognition, fabricating an appropriate chiral surface is critical and essential. It has been reported that *N*-carbobenzoxy-L-aspartic acid, a derivation of L-glutamic acid, can be used as a chiral selector to recognize aspartic acid enantiomers using Cu(II) as central ions based on chiral ligand exchange [26]. L-methotrexate (L-Mtx) has been used in the therapeutic treatments of a variety of neoplastic and proliferative diseases [27]. As another derivation of L-glutamic acid, here, L-Mtx was chosen as a chiral selector to recognize Pen enantiomers. The interaction between L-Mtx and Pen enantiomers in the absence or in the presence of Zn(II) were comparative studied via cyclic voltammetry.

Materials and methods

Reagents and materials

L-Mtx was purchased from J&K technology Co., Ltd. (shanghai, china). *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimidehydrochloride (EDC), *N*-hydroxy succinimide (NHS), L-penicillamine, and D- penicillamine were bought from Sigma chemical Co. (St. Louis, MO, USA). 3-mercaptopropionic acid (MPA) was obtained from Koch-Light Laboratories Ltd, (Coinbrook, Bucks, UK). Zn(OOCCH₃)₂ and all other reagents were commercially available and analytical grade. Distilled water was used throughout the experiments. Phosphate-buffered saline (PBS; pH 5.4) was prepared by 0.1 M Na₂HPO₄ and 0.1 M KH₂PO₄ containing 0.1 M KCl as the supporting electrolyte.

Apparatus

Electrochemical impedance spectroscopy (EIS) measurements were performed on IM6e (ZAHNER Elektrick Co., Germany) and cyclic voltammetric (CV) measurements were carried with a CHI 660D electrochemistry workstation (Shanghai Chenhua Instruments Co., China). All experiments were performed with a conventional three-electrode system. The bare or modified electrode as working electrode, a platinum wire or a saturated calomel electrode as auxiliary electrode and reference electrode, respectively. All measurements were performed at room temperature.

Preparation of L-Mtx self-assembled gold electrodes

The gold electrodes ($\Phi=3$ mm) were polished carefully with 1.0, 0.3, and 0.05 μm alumina slurries, respectively. Then

the electrodes were sonicated in double-distilled water, ethanol, and double-distilled water each for 5 min. Following that, the cleaned electrodes were immediately immersed in 5 mM MPA ethanol solution overnight at 4 °C, forming a self-assembling monolayer with free carboxyl groups through Au-S bond [28]. Thereafter, the modified electrodes were rinsed with absolute ethanol to remove physical adsorbed MPA molecules. Subsequently, the carboxyl group of the MPA was activated by immersing the electrodes in an aqueous solution containing EDC and NHS for 1.5 h. At last, the electrodes were immersed in 0.1 mM L-Mtx about 7 h, and the L-Mtx-modified gold electrode (L-Mtx-Au) was obtained. The stepwise preparing of the modified electrodes is shown in Scheme 1

Experimental measurements

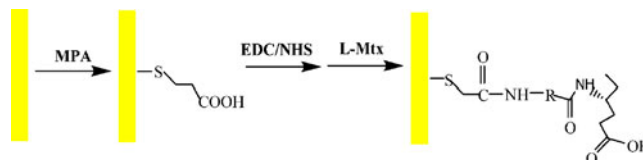
The CV scans were performed from -0.2 to 0.6 V at $0.1 \text{ V}\cdot\text{s}^{-1}$ in 5 mM [Fe(CN)₆]^{4-/3-} (pH 5.4, 0.1 M PBS). EIS measurements were done in 5 mM [Fe(CN)₆]^{4-/3-} solution too. The enantioselective detection was based on the difference of decrease of peak current (ΔI) before and after the modified electrode immersed in L-Pen or D-Pen in the presence of Zn(II) (L-Pen + Zn(II) and D-Pen + Zn(II)). ΔI was given by the following equation: $\Delta I = I_1 - I_0$, where I_1 represents the peak current of the modified electrode, and I_0 was regard as the peak current after the modified chiral surface interacted with analyte.

Results and discussion

Characteristics of the L-Mtx modified electrode

The adsorption quantity of MPA on gold electrode was analyzed by the CVs of MPA-coated gold sensors in the 0.5 M KOH solution from -0.4 to -1.2 at $100 \text{ mV}\cdot\text{s}^{-1}$. The molecular density (Γ) of MPA on the gold surface was $1.26 \times 10^{-10} \text{ mol}\cdot\text{cm}^{-2}$ [11] (see Fig. S1 in Supplemental materials).

The CV characterization of stepwise self-assembly was shown in Fig. 1. A standard redox peak was found on the bare gold electrode (curve a). The peak current was decreased after MPA was absorbed onto the electrode surface (curve b) owing to the MPA greatly obstructs electron and



Scheme 1 The schematic illustration of the chiral surface fabrication process **a** bare Au, **b** MPA/Au, **c** L-Mtx/MPA/Au

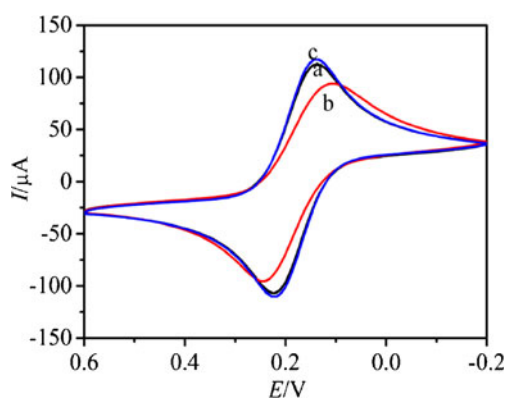


Fig. 1 Cyclic voltammograms of different electrodes in 5 mM $[\text{Fe}(\text{CN})_6]^{4-/3-}$ at $100 \text{ mV}\cdot\text{s}^{-1}$ **a** bare Au, **b** MAP/Au, **c** L-Mtx/MPA/Au

mass transfer. The peak currents presented an obvious increase when the electrode was modified with the L-Mtx (curve c), implying the L-Mtx modified electrode catalyzed the redox behavior of the $[\text{Fe}(\text{CN})_6]^{4-/3-}$ couple.

The stability of the chiral surface was investigated. An *RSD* of 0.27% was acquired after 50 CV cycles in 5 mM $[\text{Fe}(\text{CN})_6]^{4-/3-}$, indicating sufficient stability of the L-Mtx modified electrode. The CVs of the prepared chiral surface at different scan rates were also considered. The redox peak currents were proportional to the square root of scan rate ($v^{1/2}$) from 20 to $500 \text{ mV}\cdot\text{s}^{-1}$, suggesting a diffusion controlled electron transfer process [29] (see Fig. S2 in Supplemental materials).

Enantioselective recognition of penicillamine by L-Mtx-Au

Investigation of the interaction between L-Mtx and Pen enantiomers

The interaction between L-Mtx-Au and Pen enantiomers was investigated by CV. Insert of Fig. 2a showed the electrochemical response before and after the modified electrode interacted with Pen enantiomers. After L-Mtx-Au reacted

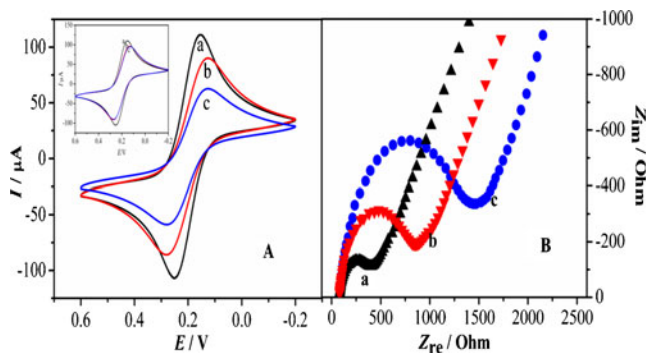


Fig. 2 **a** CVs and **b** EIS of **a** L-Mtx-Au and after L-Mtx-Au interacted with **b** L-Pen + $\text{Zn}(\text{II})$ or **c** D-Pen + $\text{Zn}(\text{II})$ about 15 min in 5 mM $[\text{Fe}(\text{CN})_6]^{4-/3-}$. Insert of **a**: CVs of **a** L-Mtx-Au and after L-Mtx-Au interacted with **b** L-Pen or **c** D-Pen for 15 min

with Pen certain time, the peak current changed a little (curve b and c), attributing to H-bond or electrostatic forces. But the changes of peak current both L-Pen and D-Pen were almost the same. It can be concluded that there was no difference of electrochemical signal in the interaction between L-Mtx-Au and Pen enantiomers.

Investigation of the interaction between L-Mtx and Pen enantiomers in the presence of $\text{Zn}(\text{II})$

It is well known that $\text{Zn}(\text{II})$ could form metal complexes with amino acid through amino group and carboxy group, which can be used in enantioselective recognition [30]. In our previous study, we observed highly enantioselective recognition of mandelic acid in the presence of $\text{Zn}(\text{II})$ using L-Cys-Au [31]. In this study, $\text{Zn}(\text{II})$ was chosen as central ions for the selective recognition of Pen enantiomers.

The interaction between L-Mtx and Pen enantiomers in the presence of $\text{Zn}(\text{II})$ was investigated by CV. As shown in Fig. 2a, a good redox peak of $[\text{Fe}(\text{CN})_6]^{4-/3-}$ couple was observed on L-Mtx-Au (curve a). The peak current of the modified electrode decreased after it interacted with the mixture of penicillamine and $\text{Zn}(\text{II})$ for 15 min, suggested the L-Mtx could interact with Pen in the presence of $\text{Zn}(\text{II})$ and introduce a barrier on the electrode surface for electron transfer. It could be noticed that the ΔI_D (curve c) was larger than ΔI_L (curve b). That is to say, L-Mtx-Au may selectively interact with Pen enantiomers in the presence of $\text{Zn}(\text{II})$ and cause different electrochemical response signals. The average difference of ΔI_D and ΔI_L was $31.34 \mu\text{A}$, and the relative standard deviation (*RSD*) was 3.9%, $n=6$.

EIS is an effective tool for probing the interface properties of surface-modified electrode [32]. The semicircle diameter of EIS equals to the electron-transfer resistance (R_{et}). In this work, EIS was also used to measure the enantioselective interaction between Pen enantiomers and L-Mtx in the presence of $\text{Zn}(\text{II})$. As shown in Fig. 2b, compared with L-Mtx-Au (curve a), the impedance increased after the L-Mtx-Au interacted with L- or D-Pen in the presence of $\text{Zn}(\text{II})$ for 15 min and the impedance of D-Pen was larger than L-Pen, suggesting larger interfacial resistance was obtained from D-Pen. It may be concluded that L-Mtx-Au could enantioselective recognize Pen enantiomers in the presence of $\text{Zn}(\text{II})$, which is in concordance with CVs.

Time dependency of the interaction between L-Mtx-Au and Pen enantiomers

The effect of interaction time between L-Mtx and L- or D-Pen + $\text{Zn}(\text{II})$ on the current response was considered. As shown in Fig. 3a, the electrochemical response signal was obviously difference after L-Mtx-Au interacted with Pen

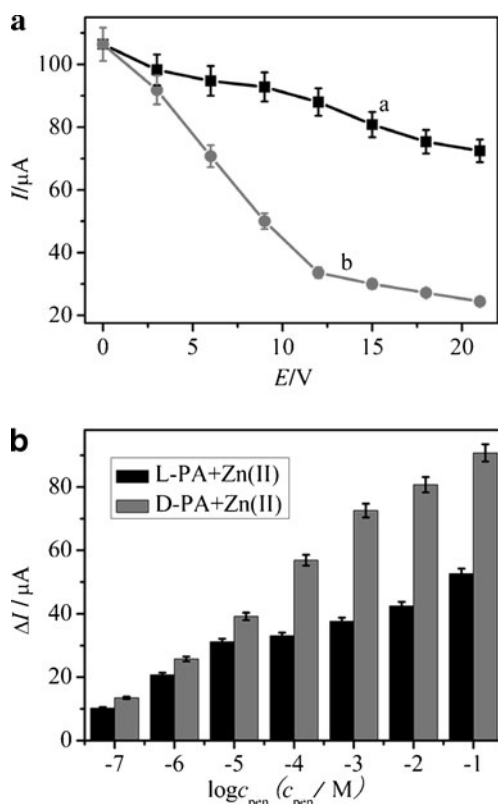


Fig. 3 **a** Effect of interaction times on the current response when L-Mtx-Au inserted in Pen + Zn(II); **a** L-Pen + Zn(II), **b** D-Pen + Zn(II). The concentration of Pen and Zn(II) was 2 and 0.03 mM, respectively. **b** The current response of L-Mtx-Au to different concentration of Pen + Zn(II) in 5 mM $[\text{Fe}(\text{CN})_6]^{4-/3-}$ at 100 $\text{mV}\cdot\text{s}^{-1}$; black L-Pen + Zn(II), gray D-Pen + Zn(II)

enantiomers in the presence of Zn(II). The difference of peak current both D- and L-Pen were increased with the prolongation of interaction time because of a longer reaction time contributed to more Pen + Zn(II) molecules interacted with the modified electrode surface, and the ΔI_D always higher than ΔI_L . The changes of response signal became small after interacted about 15 min. Therefore, 15 min was chosen the reaction time.

The electrochemical response of the modified electrode to a series of concentration of Pen from 0.1 μM to 0.1 M in the presence of 0.03 M Zn(II) was also investigated by measuring the change of peak current. As shown in Fig. 3b, the ΔI_D were bigger than the ΔI_L , and the difference of ΔI_D and ΔI_L gradually increased with the increasing of L-Pen and D-Pen concentration. It was notice remarking that the difference of electrochemical signal can be observed even 0.1 μM Pen enantiomers and the change of peak current has a linear relation with concentration of L-Pen and D-Pen. It is further evidence that the L-Mtx-Au can selectively interact with Pen enantiomers in the presence of Zn(II).

All above results show that there was no difference of electrochemical response for Pen enantiomers using L-Mtx-

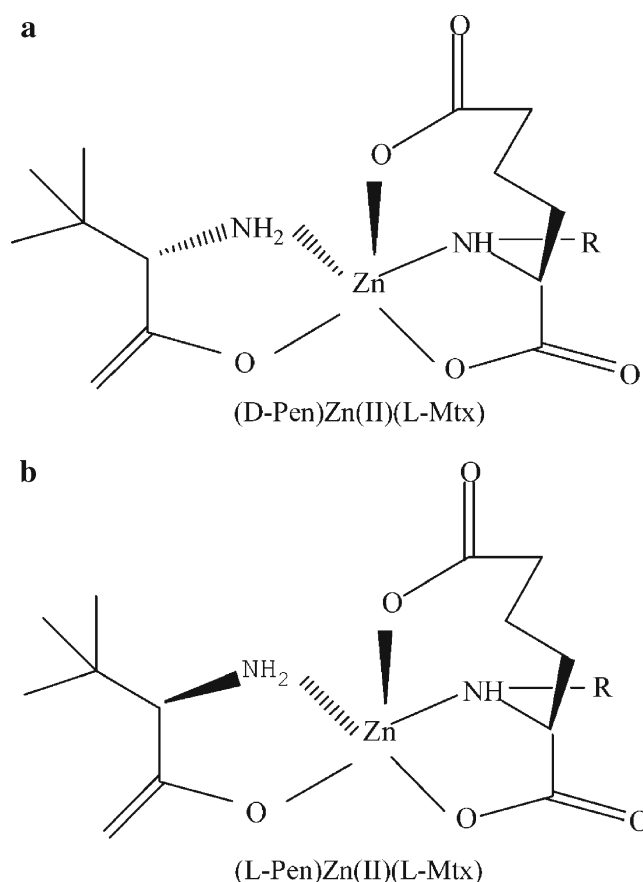


Fig. 4 Proposed chemical structures of mixed ternary complexes of L-Mtx with **a** D-Pen and **b** L-Pen

Au, while larger difference was obtained in the presence of Zn (II). It shows that Zn(II) played an extraordinary important role in this enantioselective recognition. Penicillamine contain

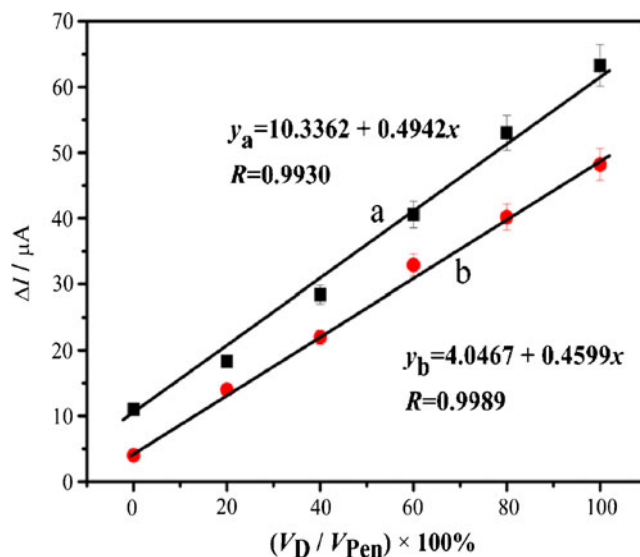


Fig. 5 The decrease of peak currents after the L-Cys-Au interacted with enantiomeric composition of L-Pen and D-Pen in the presence of Zn(II) at different concentrations: **a** 5 mM and **b** 2 mM

carboxyl group and amino group which can form ligand complexes with Zn(II). After $\text{Zn}(\text{OOCCH}_3)_2$ was added into L- or D-Pen solution, $[\text{Zn}(\text{II})(\text{L-Pen})_2]$ or $[\text{Zn}(\text{II})(\text{D-Pen})_2]$ was formed [33]. Under suitable conditions, the chiral selector L-Mtx will exchange with L- or D-enantiomers of the analyte penicillamine in the presence of Zn(II) to form the ternary complexes composed of the selector, central Zn(II) and penicillamine. When L-Mtx-Au inserted in $[\text{Zn}(\text{II})(\text{L-Pen})_2]$ or $[\text{Zn}(\text{II})(\text{D-Pen})_2]$ solution, the formation of $[(\text{L-Mtx})\text{Zn}(\text{II})(\text{L-Pen})]$ or $[(\text{L-Mtx})\text{Zn}(\text{II})(\text{D-Pen})]$ may introduce a barrier on the electrode surface for electron transfer and different barrier was obtained, attributing to the different optical isomer of D- and L-Pen which may cause the difference of the formation of Zn complexes. Figure 4 showed the imaginable chemical structures of mixed ternary complexes of L-Mtx with L-Pen and D-Pen. From the structures of mixed ternary complexes, it can be seen that the formation of $[(\text{L-Mtx})\text{Zn}(\text{II})(\text{D-Pen})]$ diastereoisomeric complexes is more easy than $[(\text{L-Mtx})\text{Zn}(\text{II})(\text{L-Pen})]$ complexes, suggesting the molecular configuration of D-Pen match better with L-Mtx, and induce a weaker steric hindrance during the formation of Zn complex $[(\text{L-Mtx})\text{Zn}(\text{II})(\text{D-Pen})]$. As stated above, the enantioselective recognition of chiral Pen using L-Mtx-Au is resulted from the enantioselective forming Zn complexes with L-Mtx and Pen enantiomers.

Application of the modified electrode

The modified electrode was also used to measure the current response of a series of solutions which were prepared by mixing L-Pen and D-Pen at different fixed ratios in the presence of Zn(II). The difference of the relative peak currents along with the proportions of enantiomers is shown on Fig. 5. It is clear that the enantiomeric composition of L-Pen and D-Pen enantiomer mixture can be determined from the calibration curves which show good linearity.

Conclusions

In this work, a new chiral sensor was fabricated through chemical linkages L-Mtx onto gold electrode which could selectively recognize Pen enantiomers with Zn(II) as central ions. This results showed that Zn(II) played an very important role in the chiral recognition of Pen enantiomers using L-Mtx-Au and larger electrochemical signal was obtained from $[(\text{L-Mtx})\text{Zn}(\text{II})(\text{D-Pen})]$ complexes than $[(\text{L-Mtx})\text{Zn}(\text{II})(\text{L-Pen})]$. The chiral recognition was based on the formation of Zn complexes. The advantage of this work is a new chiral surface was obtained and used to recognize Pen enantiomers. Larger difference of electrochemical signal was obtained from Pen enantiomers.

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